THE CHEMICAL INVESTIGATION OF SOME POISONOUS PLANTS IN THE N.O. SOLANACEÆ.

Part v.—The Alkaloids of *Duboisia Leichhardtii* F.v.M.

By James M. Petrie, D.Sc., F.I.C., Linnean Macleay Fellow of the Society in Biochemistry.

(From the Physiological Laboratory of the University of Sydney.)

Duboisia Leichhardtii is a small evergreen tree, endemic in Eastern Australia. It was discovered by Leichhardt in his travels, and sent by him to Baron von Mueller, who described it in 1867. Leichhardt's specimens are in the Melbourne Herbarium, but no locality is given other than "extra-tropical Eastern Australia." The first specimens bearing a definite locality were obtained from Mt. Playfair, near Springsure in Central Queensland. These were sent by a squatter to von Mueller in 1890, and are also in the Melbourne Herbarium. In the same year, it was found by Dr. J. Shirley growing along the Stuart River, 140 miles north-north-west of Brisbane, and specimens were placed in the Queensland National Herbarium. The only other record of this plant is that in Moore and Betche's Flora of New South Wales, where it is stated to have been found in the Gray Ranges, which cross the extreme north-west corner of New South Wales into Queensland. But there are no corresponding specimens from this locality in Australian collections.

Though so very little is known, and the records are so few, concerning *Duboisia Leichhardtii*, the three localities given lie in a great inverted crescent, 800 miles long, stretching across Queensland from the south-west corner, through the central area, and down to the south-east.

Von Mueller described the plant under the name of Anthocercis, in his Fragmenta phytographia (Vol. vi., 1867-8, p.142),

and ten years later the name was transferred to Duboisia (Wing's Southern Science Record, ii., 1882, 222). Of the three described species of Duboisia, *D. Hopwoodii* is confined to central Australia, *D. myoporoides* extends along the eastern coastline, and *D. Leichhardtii* apparently occupies the intervening country, which joins up the areas occupied by the other two species.

Although in their essential features they exhibit fundamental differences which characterise them as distinct species, they possess a peculiar similarity in their general aspect, and the writer, familiar with the species *D. myoporoides* only, had no difficulty in recognising *D. Leichhardtii* when first seen in the forests of central Queensland. In some respects *D. Leichhardtii* may be regarded as intermediate between the other two, for example, in the average heights of adult trees—*D. Hopwoodii* 8 feet, *D. Leichhardtii* 15 feet, and *D. myoporoides* 25 feet; or in the relative sizes of their mature leaves—2, 3, and 4 inches in length respectively. On the other hand, in comparing the details of the original botanical descriptions, one can hardly say that *D. Leichhardtii* inclines towards one or the other species. It is differentiated chiefly by the flowers possessing long, acute corolla-lobes. Much interest was aroused by the speculation as to whether, in regard to its active principle, this third species would resemble *D. Hopwoodii* or *D. myoporoides*, or differ from both. We have seen (Part iv. of this series) that *D. Hopwoodii*, the pituri plant, contains nicotine, and that *D. myoporoides*, the cork-tree, contains hyoscyamine and nor-hyoscyamine. This point was soon settled, but only after the completion of the investigation, a casual reference was found in a Medical Journal, to an account of some tests by Lauterer, of Brisbane, (Aust. Med. Gaz., xiv., 1895, 457) which he made on the alkaloids of *Duboisia myoporoides*. He states that, in this plant, he found hyoscyamine and scopolamine, and that "*D. Leichhardtii* contains mostly scopolamine." With the exception of this statement of a single line, no other information has been found regarding the chemical constituents. It is doubtful whether Lauterer ever worked on *D. Leichhardtii* at all; if he did, it was wholly unknown to his

friends of the Royal Society of Queensland (private communication from Dr. J. Shirley).

The results which Lauterer entirely depended upon for his conclusion in the case of *D. myoporoides* were obtained from qualitative tests only, and chiefly Gerrard's mercuric chloride reaction. The author, in repeating these tests with pure alkaloids, obtained results which did not agree with the statements of Gerrard (Pharm. Journ., xxi., 1891, 898). For instance, pure atropine and hyoscyamine were dissolved in chloroform and evaporated on a watch-glass. A 2 per cent. solution of mercuric chloride in 50 per cent. alcohol was then added drop by drop, when a yellow precipitate was obtained in both cases. These remained yellow for some hours, though they became red on heating. Atropine is stated by Gerrard to be distinguished from hyoscyamine by giving a red colour at once, without heat. Scopolamine was found to give a white precipitate, and nor-hyoscyamine also gave a white precipitate when tested in the same manner. In this way certainly hyoscyamine may be distinguished from scopolamine when separate; but, in a mixture of alkaloids, the observation of a white precipitate in Gerrard's reaction does not justify the conclusion that only scopolamine is present. The author obtained a white precipitate in the mixture of alkaloids obtained from *Solandra longiflora* (Part iii. of this series) in which no scopolamine was found, but which contained hyoscyamine, nor-hyoscyamine, and atropine.

Source of the Material.

The material for this investigation was collected in Queensland by the author, accompanied by Mr. C. White, Assistant Government Botanist. The starting point for the Duboisia country was the North-West Railway terminus at Nanango, and directions had been obtained from Dr. Shirley, who discovered the trees in this region in 1890. Far up the Stuart River and about 10 miles from Taabinga cattle-station, the first specimens of *D. Leichhardtii* were seen; and after two days' driving through open forest-country, they were still observed stretching away to the south-west towards the Bunya Bunya Mountains.

As far as can be ascertained, they are not found south of the mountains which form the watershed of the Brisbane Valley.

The trees were always observed in small clusters, growing on red volcanic soil, and only on the edge of the thin scrubs in the open brush forests.

They were invariably associated with certain prominent ironbark and acacia trees, among which the following species were noted:—*Eucalyptus crebra* (narrow-leaved ironbark), *E. paniculata* (white ironbark), *E. melanophloia* (silver-leaved ironbark), *E. tereticornis* (red gum), *E. hemiphloia* (gum-top box); *Angophora lanceolata* and *A. subvelutina*. Among the acacias were *A. penninervis*, *A. Cunninghamii*, *A. implexa*, a variety of *A. decurrens*, and *A. aulacocarpa*.

Experimental.

(i.) *Preliminary Examination for Alkaloids:*—A small quantity of the material was extracted in a Soxhlet extraction-apparatus and treated by the Stas-Otto process for the separation of active principles. A substance was obtained which gave positive reactions with the following reagents:—Iodine in potassium iodide, potassium mercuric iodide, phosphomolybdic, picric, tannic, and phosphotungstic acids, platinic and auric chlorides.

The solution possessed an intensely bitter taste and alkaline reaction.

When diluted to 1 in 1000 with normal saline and instilled into the eye of a dog, wide dilatation was produced in about 30 minutes.

It gave a strong positive reaction with Vitali's test.

The substance is thus shown to be an alkaloid of the atropine group; and since the aurichloride salts were observed under the microscope to consist of several kinds of crystals, the probability is that they contain a mixture of associated alkaloids of the midriatic group.

(2.) *Distillation for Volatile Constituents:*—About 100 gms. of air-dried leaves were powdered and mixed with milk of lime in a large flask. The mass was distilled in a current of steam

for seven hours. The alkaline distillate was shaken out with ether, and this ethereal liquid, after separating and drying, was distilled at a low temperature to dryness. This residue was dissolved in water and titrated, when it required 23 ccs. of centi-normal acid to neutralise. The fluid was then acidified and shaken out with ether, when about 11 mgs. of an oily substance were separated. On making faintly alkaline with ammonia and agitating with chloroform, a substance was removed which was afterwards obtained as a viscous residue. This weighed about 55 mgs., which is equivalent to 8 mgs. per hour. It gave precipitates with all the alkaloidal reagents, and also the characteristic Vitali's reaction for the atropine group; while a solution in normal saline 1 in 1000, widely dilated the eye of a dog in about 20 minutes.

It is apparent from these results that a minute quantity of the atropine alkaloids has distilled over, as has already been proved by the author to take place with other plants containing alkaloids of this group (Part iv. of this series).

(3.) *Extraction of the Alkaloids:*—The air-dried leaves containing 9·7 per cent. of water were ground to a fine powder, and extracted with cold methylated spirit. At weekly intervals, the latter was removed until only traces of alkaloid were dissolved. The voluminous alcoholic extracts, obtained by draining and pressing the material, were distilled under reduced pressure, and below 60°C., when there remained a dark-coloured viscous residue. This was removed by washing with successive small quantities of warm water slightly acidulated, and filtered. For the removal of colouring matter and resins, this dark brown fluid was next treated with lead acetate. The lead precipitate was carefully washed free from alkaloid, and the aqueous fluid and washings freed from lead. The solution, now only slightly coloured, was concentrated at 60°C. to a small volume. Wagner's iodine reagent was then used to precipitate the alkaloids. This iodine precipitate was decomposed by sulphurous acid, and the solution was shaken out with ether. This solvent removed a considerable amount of impurity. The aqueous solution was next made

alkaline with a very slight excess of ammonia, which separated the alkaloids in a dense white precipitate. The alkaloids were then removed in solution by agitating repeatedly with equal volumes of chloroform. The chloroform was removed by distilling under diminished pressure, when there was left in the flask a semi-solid mass, which, after standing some days, crystallised in beautiful white radiating needles.

The original plant-material was not treated in one large bulk, but in a number of small portions as required. In one of these, which yielded the maximum weight of alkaloids, the following data were recorded:—300 gms. of air-dried plant extracted for 24 days altogether. The aqueous extract was concentrated to 350 c.cs., and precipitated with one litre of Wagner's decinormal iodine solution. Ether removed from this acid solution about 2 gms. of impurities, and chloroform from the alkaline liquid yielded 5 gms. of crude alkaloid. A portion of the latter in solution was titrated with decinormal acid and iodeosin indicator, and the result showed that 81 per cent. consisted of pure alkaloid. The yield was, therefore, 1·42 per cent. of alkaloids in the dried (at 100°C.) plant-leaves, or 0·28 per cent. in the fresh plant.

The optical activity of a solution of this crude alkaloid in 50 per cent. alcohol was determined, $[\alpha]_D -18°$.

(4.) *Separation of the Alkaloids:*—The mixed alkaloids were converted directly into aurichlorides, by the addition of gold chloride to the solution of alkaloids in dilute hydrochloric acid. The yellow precipitate was dissolved in sufficient warm water, and set aside to slowly crystallise spontaneously. At regular intervals, the crystals were removed by decanting the superfluid. These crystals were washed and the melting-points determined, then redissolved in dilute hydrochloric acid and again set aside to crystallise. After a long and tedious process of fractional crystallisation, the various fractions being placed together, or separated, according to their melting-points, the latter were observed to concentrate near certain definite temperatures. These fractions were finally obtained with melting-points which did not change after further recrystallisation. A summary is

given in the following table of only the principal stages in the separation of these salts.

Fractional Crystallisation of the Aurichloride Salts.

a
1. Brown viscous deposit.
2. Yellow crystals, m.p. 135-178°.
3. Amorphous yellow mass.

b
a1 was washed and found to be non-alkaloidal.
a2 was recrystallised.
a3 was decomposed and the alkaloids recovered by shaking out with chloroform. The gold salts were then reformed and crystallised.

c
(a2+a3) when recrystallised gave (1) yellow crystals, m.p. 174-179°
(2) ,, ,, 136-162
(3) ,, ,, 180-190
(4) unerystallisable portion.

d
c1 when dissolved and recrystallised yielded 1. 177-179° 2. 135 small amt.
c2 ,, ,, ,, 3. 165-167 greater part. 4. 135-150
c3 ,, ,, ,, 5. 197-199 greater part. 6. 186

e
d1 when dissolved and recrystallised yielded (1) 178, 179, 179°
d3 ,, ,, ,, (2) 165-166
d2+d4 ,, ,, ,, (3) 137, 137
d5 ,, ,, ,, (4) 198, 198, 197

f
e1 when dissolved and recrystallised yielded 179, 179, 179°
e2 ,, ,, ,, 165-166
e3 ,, ,, ,, 137, 136, 137
e4 ,, ,, ,, 197-198

(5.) *Identification of the Alkaloids.*—The most recent work on the fractional crystallisation of the midriatic alkaloids is that of Carr and Reynolds (J.C.S., ci., 1912, 950). These authors give the following figures for the melting-points of the salts of the pure alkaloids :—

Atropine aurichloride	...	... m.p.137-139°
Lævo-hyoscyamine aurichloride		... m.p.165°
Nor-hyoscyamine	,,	... m.p.178-179°
Lævo-scopolamine	,,	... m.p.198°

A portion of the pure aurichloride crystals thus obtained were then converted into picrates, by boiling their solutions with

sulphurous acid, filtering off the deposit of metallic gold, and treating with a saturated solution of picric acid. These were allowed to stand, the crystals were separated, recrystallised, washed and dried, and their melting points taken.

(1.)	Aurichloride	m.p.179°C.	yields	picrate	with	m.p.219-220°C.
(2.)	,,	165	,,	,,	,,	163-166
(3.)	,,	136	,,	,,	,,	176
(4.)	,,	197	,,	,,	,,	180

The above authorities have given the melting points of the picrates of the pure alkaloids as follows:—

Atropine picrate... ...	... m.p.175-176°C.
Lævo-hyoscyamine picrate	... m.p.165
Nor-hyoscyamine ,,	... m.p.220
Lævo-scopolamine ,,	... m.p.180-181

From one gram of the crude alkaloid there was obtained 0·5 gm. of crystals of the gold salts. This quantity at the end of the fractional crystallisation yielded approximately 0·1 gm. of scopolamine salt, 0·1 gm. of nor-hyoscyamine salt, and 0·2 gm. of lævo-hyoscyamine salt. In other experiments, larger amounts were obtained, but considerable proportions of a viscous uncrystallisable substance always separated.

In the initial stages of the above crystallisation process (a, b, and c), when the crystals had been separated as much as possible, the mother-liquors deposited yellow, amorphous, sticky particles. When the solution was gently warmed, the amorphous substance melted and floated on the surface like oily drops. This portion, which contained part of the alkaloids, could not be induced to crystallise, and further attempts to purify it did not alter its viscous nature.

Summary.

The leaves of *Duboisia Leichhardtii* contain a mixture of the midriatic alkaloids, amounting to 1·4 per cent. of the dried (at 100°C.) material or 0·28 per cent. of the fresh plant. By the fractional crystallisation of their aurichlorides, the mixed alkaloids were separated into nor-hyoscyamine, lævo-hyoscyamine,

lævo-scopolamine, and small amounts of atropine and nor-atropine. These were identified by the melting points of their gold salts and of the picrates.

D. Leichhardtii, therefore, closely resembles *D. myoporoides* in its alkaloids, and these two species are in marked contrast to the only other species known—*D. Hopwoodii*, the pituri-plant, which contains nicotine.

The thanks of the author are due to Sir Thomas Anderson Stuart, in whose laboratory this work was carried out.